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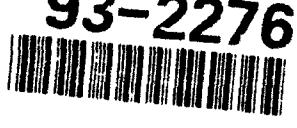

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Synthesis and Antimalarial Activity of Some 9-Substituted Artemisinin Derivatives

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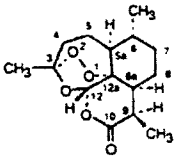
Several 9-substituted derivatives of the antimalarial drug artemisinin have been prepared by functionalizing the double bond of artemisitene and related compounds. Stereochemical assignments for these compounds were made using a combination of NMR experiments, an X-ray diffraction study of one compound, and chemical correlations of several other compounds with this one compound of unambiguous structure and with its epimer. The compounds synthesized show a wide variation in *in vitro* antimalarial activity.

Artemisinin, 1, and a number of its derivatives are under clinical study as antimalarial drugs by agencies such as the World Health Organization and the U.S. Army and by other groups both here and abroad.[1,2] Artemisinin was first isolated in China, and the Chinese and others have prepared a large number of derivatives of this sesquiterpene endoperoxide. The syntheses, chemistry, and biological activity of 1 and related compounds have recently been reviewed.[3,4] Except for those prepared by total synthesis or semisynthesis, the vast majority of these derivatives involves variation at position 10, the only position in 1 subject to straightforward chemical reaction without degradation of the peroxide functionality. A number of years ago, one of us (N.A.) isolated a closely related sesquiterpene, artemisitene, 2, from *Artemisia annua*,[5] the same plant that produces artemisinin. This compound was available in only very small amounts until 1990 when El-Feraly et al. reported a procedure for converting 1 into 2 in four high-yield steps.[6] The ready availability of artemisitene has led us to synthesize a number of artemisinin and related derivatives that are functionalized in the 9 position. All syntheses started from either artemisitene, the hydroperoxide 3, which is the last intermediate in the artemisitene synthesis,[6] or the alcohol 4 derived from 3 using a variation of the literature procedure.[7]

Compounds 5–17 were synthesized by standard procedures. Table I summarizes the starting materials and reagents used and the melting point and yield of each derivative. All derivatives were characterized by elemental analysis and ^{1}H NMR, ^{13}C NMR, and IR spectroscopy. NMR peak assignments were made using COSY, HETCORR, DEPT, and NOESY experiments. Table II is a collection of ^{13}C NMR data for these compounds and, for comparison, of artemisinin[8] and artemisitene. Table III summarizes the ^{1}H NMR peaks at positions 8–12 for each compound. The ^{1}H NMR resonances of the remaining protons are almost identical with those of artemisinin. It is noteworthy that attempted silica gel chromatography lowered the yields of some of the products. This was the case for 8, 11, and 12. Attempted epoxidations of compounds 2–4 with a variety of standard epoxidizing reagents were unsuccessful, but the relatively new reagent dimethyldioxirane, isolated in acetone, epoxidized each in reasonably good yield. Mixtures of α and β epoxides were obtained from 2 and 3, but 4 gave a single epoxide. As with other dihydroartemisinin derivatives, however, epoxide 17 epimerizes in solution at position 10. This is also the case for compound 9.

Chart I. Structures of Artemisinin and Some Previously Reported Derivatives



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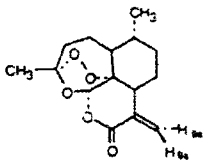

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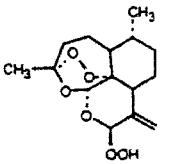

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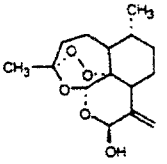

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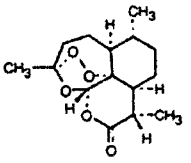

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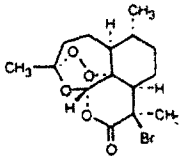

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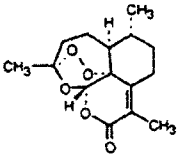

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Stereochemical assignments at position 9 are based on NOESY NMR experiments. Significant cross peaks are summarized in the Experimental Section. In addition, epoxide 14 was obtained as single crystals upon recrystallization from CH_2Cl_2/ether. The X-ray crystal structure of 14 confirms the configuration of the epoxide where the epoxide oxygen points toward the peroxide moiety (Figure 1).[9] Although two molecules of differing conformation crystallized in the asymmetric unit, the conformation of the two molecules is almost identical, except for the twist of the boat-shaped O4–C10–C9–C8a–C12a–C12 ring and the epoxide group. This is exemplified by the torsion angles C12a–C8a–C9–C10 equal to −29.0(0.8)° and −36.4(0.9)° and O6–C9–C10–O4 equal to −146.2(0.6)° and

Table I. Reaction Conditions, Melting Points, and Yields

product	starting material	reagents, condns	mp (°C, dec)	% yield
5	2	Xs CH_2N_2, 24 h, rt	138–139	75
6	5	145 °C/25 min	161–164	52
7	5	*hν*, 1 h, ca. 0 °C	174–176	35
8	2	O_3, −78 °C, then Me_2S, rt	174–176	73
9	8	BH_3, THF, rt	153–156	63
10	8	$H_2NNHCSSCH_3$, MeOH, reflux 30 min	162–165	60
11	2	NBS, *hν*, 2 days	129–130	12
12	2	cat. OsO_4, NMO, aq acetone, rt, ca. 36 h	136–137	90
13	2	dimethyldioxirane, rt, overnight separated from 14 by silica gel chromatography	180–181	33
14	2	dimethyldioxirane, rt, overnight separated from 13 by silica gel chromatography	171–173	38
15	3	dimethyldioxirane rt, overnight separated from 16 by silica gel chromatography	140	17
16	3	dimethyldioxirane, rt, overnight separated from 15 by silica gel chromatography	144–145	38
17	4	dimethyldioxirane, rt, overnight	152–154	53
13	15	Ac_2O, pyridine		
14	16	Ac_2O, pyridine		
17	16	$(EtO)_3P$		
13	12	TsCl/pyr, rt, then NaH, cat imidazole, THF		

Table II. Carbon NMR Shifts for Artemisinin Derivatives

C-atom	1[a]	2	5	6	7	8	9[b]	10	11	12	13	14	15	16	17[c]
10 s	171.9	162.7	166.7	162.2	172.3	187.8	97.4, 91.1 d	130.2	166.7	173.3	166.9	166.7	105.7 d	106.7 d	97.0, 88.4 d
3 s	105.2	105.4	105.6	105.2	105.4	106.0	104.5, 104.3	105.9	105.6	105.5	105.7	105.6	104.8	104.9	104.6, 104.3
12 d	93.6	93.5	94.3	92.7	93.7	94.3	91.1, 87.7	94.2	94.6	94.6	94.0	94.3	88.4	88.4	92.2, 86.1
12a s	79.4	79.4	79.9	79.9	80.1	81.5	82.2, 81.5	79.9	81.9	80.9	81.2	79.8	82.2	80.2	80.7, 80.5
5a d	49.9	50.1	50.4	50.1	50.3	50.1	52.1, 51.3	50.0	50.9	50.5	50.2	49.9	52.1	51.7	51.9, 51.3
8a d	44.8	46.1	50.6	48.6	47.1	52.2	45.8, 45.2	46.9	46.1	46.1	45.2	45.4	45.7	45.8	46.42, 46.39
6 d	37.4	37.7	37.4	37.8	37.7	37.6	37.2, 37.1	37.7	37.4	37.6	37.6	37.5	37.4	37.3	37.23, 37.16
4 t	35.8	35.9	35.8	36.0	35.9	35.6	36.4, 36.2	35.7	35.8	35.8	35.7	35.7	36.2	36.1	36.26, 36.15
7 t	33.5	33.7	33.6	33.8	33.8	33.1	34.3, 33.7	33.3	34.2	33.4	33.0	33.4	33.5	33.7	34.0, 33.6
5 t	24.8	24.6	24.8	24.7	24.9	24.1	24.4, 24.3	24.5	24.4	24.6	24.8*	24.5	24.3	24.6	24.5, 24.4
3-CH_3 q	25.1	25.4	25.1	25.4	25.3	25.4	26.0, 25.9	25.3	25.5	25.0	25.2	25.3	25.8	25.8	25.9, 25.9
8 t	23.3	31.6	26.0	31.8	28.2	25.9	27.3 26.3	30.0	32.7	24.0	24.6*	26.8	25.4	26.3	24.7, 26.4
9	32.8 d	135.0 s	92.9 s	126.0 s	21.0 s	153.5 s	71.5, 71.0	157.9 s	64.0 s	73.6 s	53.4 s	52.8 s	55.6 s	54.7 s	57.7, 56.5
6-CH_3 q	19.7	19.8	19.7	19.8	19.8	19.5	20.2, 20.1	19.7	19.8	19.8	19.7	19.7	20.1	20.0	20.1, 20.1
9a	12.5 q	130.3 t	34.2 t	146.6 d 9a 16.4 q 9a-CH_3	12.3 t 9a 23.7 t 9b			17.6 q SCH_3	45.4 t	69.3 t	57.1 t	50.1 t	55.7 t	47.0 t	46.9, 46.7
9b			78.5 t												

[a] Reference 8. [b] Underlined peaks belong to the major anomer resulting after equilibration in $CDCl_3$. [c] Underlined peaks belong to the initially formed major anomer.

−136.4(0.6)° for molecules A and B, respectively. The overall conformation of the fused ring systems in **14** is similar to the crystalline conformation of the artemisinin derivative 9-bromo-10-deoxy-(2-pyridylamino)artemisinin whose structure determination confirmed the absolute configuration of the artemisinin family of compounds.[10] With this one structure unambiguously determined, the chemical interconversions summarized in the last four entries in Table I secure the stereochemistry of compounds **12**, **13**, **15**, **16**, and **17**. Thus, treatment of diol **12** with *p*-toluenesulfonyl chloride afforded a monotosylate, which on treatment with sodium hydride gave epoxide **13**. Treatment of hydroperoxides **15** and **16** with acetic anhydride and pyridine afforded epoxides **13** and **14**, respectively. Treatment of hydroperoxide **16** with triethyl phosphite gave acetal **17**.

Discussion

That the substitution and stereochemistry at position 9 has an impact on antimalarial activity was evident to us in 1987 when one of us (N.A.) reported the synthesis and biological activities of 9-*epi*-artemisinin, **18**, 9-bromoartemisinin, **19**, and isoartemisitene, **20**.[11] Compounds **19** and **20** were approximately 10 times less active than artemisinin, and **18** was 50% as effective as **1** in an *in vitro* antimalarial screen. Avery and co-workers prepared 6,9-desmethylartemisinin by total synthesis in 1989, and they reported that this compound "displayed significant antimalarial activity against resistant strains of *P. falciparum*".[12] In 1991, Lin et al. reported a 5–6-fold difference in antimalarial activity for a pair of 9α- and 9β-hydroxyartemisinin derivatives.[13]

Table IV gives the *in vitro* antimalarial activities of these new compounds as well as of **2**–**4**. Activity is reported in ng/mL and is also listed in nmol/mL relative to the activity of artemisinin. These compounds were tested against clones of human malaria, *P. falciparum* D–6 (Sierra Leone clone) and W-2 (Indochina clone). The D-6 clone is a strain that is resistant to mefloquine, and the W-2 clone is resistant to chloroquine, pyrimethamine, sulfadoxine, and quinine. All but two of the new derivatives showed some activity in these assays. As is true for artemisinin itself, the active compounds do not show cross-resistance with mefloquine- or cloroquine-sensitive clones. It was reported earlier that **2** is somewhat less active than **1**.[11] Replacing the lactone carbonyl of **2** with a hydroperoxy group (compound **3**) appears to have little effect. Although conversion of artemisinin into the hemiacetal dihydroartemisinin is known to enhance activity,[3] compound **4** shows very poor activity. This result, reproduced in repeated

Table III. Proton NMR Shifts for Artemisinin Derivatives (δ in ppm)

compd	1[a]	2	5	6	7	8	9[b]	10	11	12	13	14	15	16	17[c]
proton															
8α	1.87	1.73	2.40	1.69	1.77	1.98	1.70	1.97	2.17	2.28	2.02	1.79*	1.40	1.50*	1.77
8β	1.12	1.53	1.98	1.53	1.46	1.70 ddd	1.46	1.60	1.56	1.19	1.50	1.59*	1.40	1.95*	1.65
8a	1.75	2.55 dd	1.92	2.37	1.15 dd	2.62 dd	2.08	2.68 dd	2.36	2.03	1.65	1.65	1.31	1.30	1.46, 1.35
9	3.40						3.33 dt			ca 3.4 br OH					
9a	1.21 (CH_3)d	a: 6.56 d	a, a′ 2.50, 1.55	H: 6.29 q	a: 0.75,		3.55 d (OH)		a, a′ AB q	a, a′ AB q	a, a′ AB q	a, a′ AB q	a, a′ AB q	a, a′ AB q	a, a′: AB q
				CH_3: 2.22 d	9a′: 1.50				4.27, 4.07	4.17, 3.69	3.30, 2.84	3.34, 2.80	3.02, 2.79	2.85, 2.64	2.79, 2.63
		a′: 5.67 "t"	b: 4.55 dt		b: 0.95,										
					9b′: 1.52										2.83, 2.49
		b′: 4.88 ddd													
10							4.94 dd						4.98 s	4.96 d	4.94 br dd
															5.24 d
10a							4.20 d (OH)	13.7 br s (OH)					9.50 br (OOH)	10.0 br s (OOH)	3.22 d (OH),
															3.55 d (OH)
12	5.87 s	5.99 s	6.20 s	5.92	6.02	6.26 s	5.37 s	6.08 s	6.00 s	5.94 s	6.08 s	3.09 s	5.79 s	5.81 s	5.78, 5.51 s
								2.60 s SCH_3							

[a] Reference 8. [b] Initially formed anomer. [c] Underlined peaks belong to initially formed anomer. * Assignments may be interchanged.

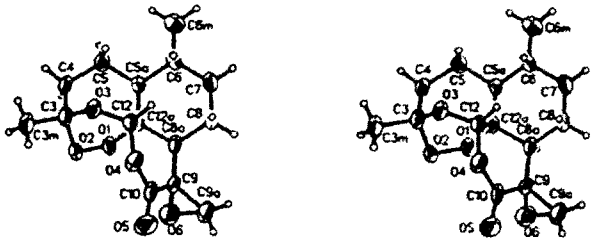
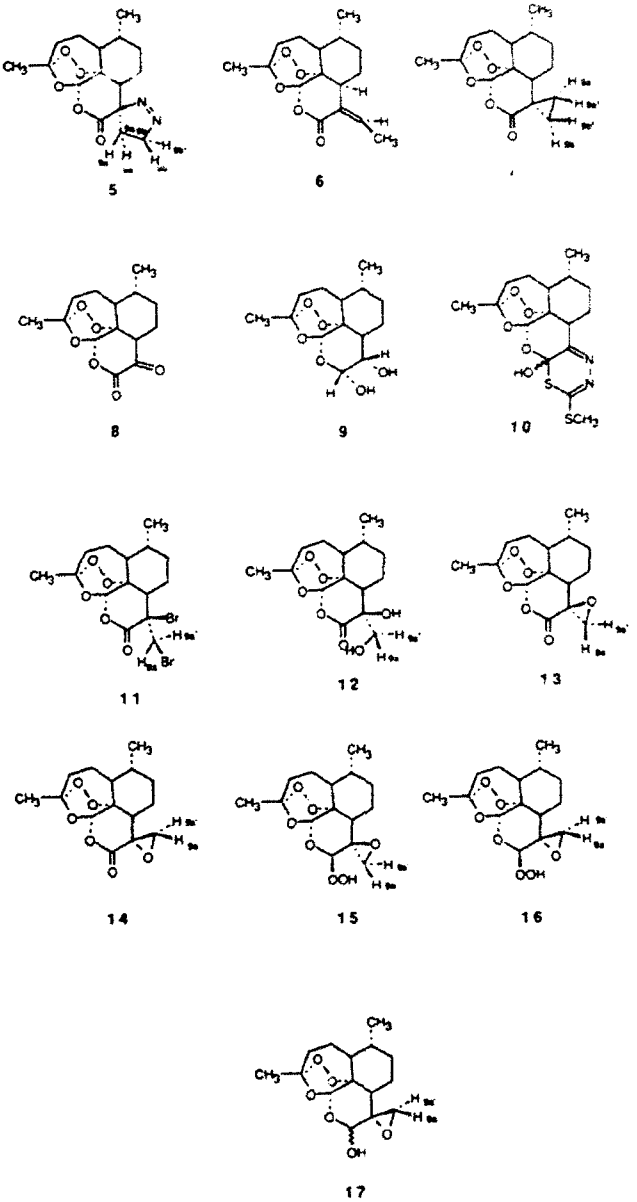


Figure 1. Stereothermal ellipsoid plot of 14 at 50% probability level drawn from the experimental coordinates of molecule A.

Chart II. New Artemisinin Derivatives



assays, is presumed to be an artifact related to the known very facile conversion of 4 into an aldehyde peroxide derivative.[7] Among the other derivatives, there is considerable variation in activity. Addition of extremely large groups (compounds 5, 10, and 11) appears to significantly reduce activity. Addition of a one-carbon extension at position 9 as in compounds 6 and 7 has little effect on activity, although 6 appears to be marginally better than 7 or 2. Replacing the 9—CH_2 group with a carbonyl as in compound 8 results in a precipitous drop in activity. The activity is restored by reducing 8 to the hydroxy hemiacetal

Table IV. *In Vitro* Antimalarial Activity of Artemisinin Derivatives against *Plasmodium falciparum*

	IC_{50} in ng/mL (IC_{50} nmol/mL relative to IC_{50} for 1)	
compd	Sierra Leone clone (D-6)	Indochina clone (W-2)
1	1.89 (1)	0.95 (1)
2	5.30 (2.8)	7.44 (7.8)
3	4.08 (2.0)	8.12 (8.0)
4	119 (63)	232 (242)
5	604 (280)	447 (408)
6	2.1 (1.1)	2.3 (2.3)
7	10.0 (5.2)	8.6 (8.6)
8	319 (169)	278 (290)
9	7.3 (3.8)	7.4 (7.6)
10	785 (305)	564 (429)
11	77 (26)	67 (44)
12	NA[a]	NA[a]
13	96 (48)	124 (123)
14	NA[a]	NA[a]
15	2.8 (1.3)	4.5 (4.2)
16	2.0 (0.9)	4.0 (3.7)
17	1.8 (0.9)	3.4 (3.4)

[a] Not active up to 5000 ng/mL.

9. Diol **12** has no discernable activity up to 5000 ng/mL. It has been suggested that increased polarity and increased water solubility is associated with decreased antimalarial activity,[14] yet artesunate, the water-soluble succinic acid half ester of dihydroartemisinin is very active.[3,4] Perhaps the most perplexing results are those for the epoxides **13**–**17**. Epoxide **13** with the epoxy oxygen in the β position has rather poor activity, whereas its α isomer **14** has none whatever. On the other hand, the hydroperoxide analogs **15** and **16** of each of these compounds have high activity, close to that of artemisinin itself. Ignoring for the moment the fact that exchanging the lactone in **2** for the hydroperoxy group in **3** has no effect (*vide supra*), it is at first tempting to ascribe the resurrected activity of **15** and **16** to the oxidizing properties of the hydroperoxide functionality.[15] However, this cannot be the explanation because converting the hydroperoxide **16** into the lactol **17** results in a compound with identical activity.

Although it is difficult to find a unifying explanation for these *in vitro* assays, perhaps an important message is that lack of activity in a parent compound need not consign derivatives of that compound to the dustbin of chemistry since changing the hybridization at position 10 can convert compounds with little or no discernable activity into derivatives with quite respectable antimalarial behavior.

Experimental Section

^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were measured in $CDCl_3$ on a Bruker AC-F 300 spectrometer. All IR spectra were determined in KBr and are reported in cm^{-1}. Melting points are uncorrected. Compounds **2** and **3** were synthesized from **1** according to the literature procedure.[6] Alcohol **4** was prepared from **3** using a variation of the literature procedure.[7]

Dihydroartemisitene, 4. A solution of triethyl phosphite (360 mg, 2.2 mmol) in 0.5 mL of benzene was added during 1–2 min to a solution of hydroperoxide **3** (550 mg 1.9 mmol) in 5 mL of benzene (exothermic). After an additional 3–5 min, the mixture was diluted with 2–3 mL of hexane and cooled briefly in ice and the flocculent crystalline product (340 mg) collected and washed with hexane. By concentrating the filtrate and then diluting with hexane, an additional 54 mg of material was obtained for a total yield of 75%, mp 126–127.5 °C (lit.[7] 120–121 °C). This material was identical with that produced using polymer-bound triphenylphosphine as described in the literature.[7] The triethyl phosphite reaction is faster and cheaper and gives a cleaner product, especially when the reaction is carried out on a >100-mg scale.

Diazomethane Adduct of 2, 5. A large excess of diazomethane (ca. 7 mmol) in 20 mL of ether was added to an ice-cooled suspension of **2** (208 mg, 0.74 mmol) in 10 mL of ether. After the mixture was stirred at room temperature for 24 h, solvent was removed, and the residue was recrystallized from CH_2Cl_2/ether/petroleum ether to afford 180 mg (75%) of product, mp 138–139 °C dec. Anal. ($C_{16}H_{22}O_5N_2$) C, H, N. IR: 1725 (C=O). ^1H NMR, Table III. The stereochemical assignment at position 9 is based on the downfield shift of 8α. H-9a′ is assigned as shown because of an NOE with H-8a. H-9a is downfield relative to 9a′ because it is in the deshielding cone of the carbonyl. H-9b and H-9b′ are assigned by an NOE between 9a and 9b and between 9a′ and 9b′.

9-Desmethyl-9-ethylideneartemisinin, 6. Azo derivative **5** (150 mg, 0.46 mmol) in 5 mL of 1,1,2,2-tetrachloroethane was heated at 145 °C for 25 min in a sealed evacuated tube. After removal of solvent, flash chromatography on silica gel (CH_2Cl_2) afforded 70 mg (52%) of **6**, mp 161–164 °C and 30 mg of compound **7**. **6**: Anal. ($C_{16}H_{22}O_5$) C, H. IR: 1714 (C=O), 1637 (C=C). ^1H NMR, Table III. The stereochemistry at 9a is assigned because of an NOE between the olefinic H-9a and H-8a.

9′-Desmethylspiro[cyclopropane-1,9′-artemisinin], 7. Azo derivative **5** (160 mg, 0.5 mmol) was dissolved in 10 mL of methanol and 1 mL of CH_2Cl_2 in a sealed Pyrex tube. The ice-cooled solution was irradiated for 1 h with a medium-pressure Hanovia mercury lamp. After removal of solvent, flash chromatography (silica gel, 2:1 hexane/ethyl acetate) afforded 51 mg (35%) of **7**, mp 174–176 °C dec. Anal. ($C_{16}H_{22}O_5$) C, H. IR: 1724 (C=O). ^1H NMR, Table III. Assignments of 9a and 9b protons are based on an apparent NOE between H-9b and H-8a.

9-Desmethyl-9-oxoartemisinin, 8. Ozone was passed for 4 min through a dry ice–acetone-cooled solution of **2** (1.00 g, 3.6 mmol) in 20 mL of CH_2Cl_2. After standing for 10 min at −78 °C, the solution was purged with nitrogen. Dimethyl sulfide (2 mL) was added and the solution let stand at room temperature for 45 min. After removal of solvent, the residual orange-brown oil was kept overnight at 0.1 mm. The resulting solid was taken up in CH_2Cl_2, clarified with charcoal, and filtered through Celite. After concentration, dilution with ether and then petroleum ether afforded 734 mg (73%) of white crystalline product, mp 174–176 °C dec. Attempted chromatography of this compound led to extensive loss of material. Anal. ($C_{14}H_{18}O_6$) C, H. IR: 1754 and 1747 (C=O's).

9-Desmethyl-9-hydroxydihydroartemisinin, 9. Excess diborane in THF (2 mL of a 1 M solution) was added by syringe to a solution of keto lactone **8** (125 mg, 0.44 mmol) in 4 mL of THF. After 1 h at room temperature, the reaction was quenched by addition of 2 mL of water. The clear solution was stirred for 1 h, and then most of the solvents were evaporated. The residue was taken up in ethyl acetate and the organic layer washed twice with brine. The aqueous layer was extracted once with ethyl acetate. The combined organic extract was dried ($MgSO_4$) and filtered, and the solvent was evaporated. The residual oil solidified on standing. It was flash chromatographed on silica gel (1:1 hexane/ethyl acetate) to yield 80 mg (63%) of **9**, mp 153–156 °C dec. Anal. ($C_{14}H_{22}O_6$) C, H. IR: 3378 (OH) and no C=O. From the ^1H NMR, this appears initially to be a ca. 1:1 anomeric mixture at H-10 which, on standing in $CDCl_3$, undergoes equilibration to give a major anomer (ca. 5:1). Peaks listed in Table III are of the major constituent of the equilibrated mixture. The underlined resonances in Table II are the ^{13}C NMR adsorptions of this major constituent. From the HETCORR NMR, it is apparent the C-10 and C-12 are superimposed. The stereochemsitry at position 10 is based on a cross peak in the NOESY spectrum between H-12 (β) and H-10. The stereochemistry at position 9 is based on a small NOESY cross peak between H-10 and H-9.

Methyl Hydrazinocarbodithioate Adduct of 9-Desmethyl-9-oxoartemisinin, 10. Methyl hydrazinocarbodithioate (60 mg, 0.5 mmol) was dissolved in 2.5 mL of warm methanol. Keto lactone **9** (140 mg, 0.5 mmol) was added and the yellow solution warmed to reflux for 30 min. Yellow crystalline product precipitated from the hot solution. After cooling, the product was collected and washed with cold methanol, 114 mg (60%), mp 162–165 °C dec. Anal. ($C_{16}H_{22}N_2O_5S_2$) C, H, N, S. IR: 1693 m (C=N), 3400 (OH).

9,9a-Dibromoartemisinin, 11. A sealed tube containing artemisitene (140 mg, 0.5 mmol) and *N*-bromosuccinimide (300 mg, 1.7 mmol) in 8 mL of CH_2Cl_2 was irradiated at ambient temperature with a Westinghouse street lamp for a total of 16.5 h over 2 days. After filtration and removal of solvent, the crude product was flash chromatographed twice on silica gel (2:1 hexane/ethyl acetate) and then recrystallized from ether/petroleum ether to yield 27 mg (12%) of **11**, mp 129–130 °C dec. A considerable amount of this product is lost on silica gel chromatography. Anal. ($C_{15}H_{20}O_5Br_2$) C, H, Br. IR: 1742 (C=O). ^{1}H NMR: The stereochemistry shown was assigned on the basis of a small NOESY cross peak between H-8a at 2.36 and H-9a′ at 4.07.

9,9a-Dihydroxyartemisinin, 12. A solution of artemisitene (280 mg, 1.0 mmol) in 4 mL of acetone was added to a solution of NMO (180 mg, 1.5 mmol) and OsO_4 (1 mL of a solution prepared from 1 g of OsO_4 + a trace of *tert*-butyl hydroperoxide in 50 mL of 2-methyl-2-propanol) in 2 mL of acetone + 3 mL of water. After the solution was stirred overnight, reductive electrochemical liquid chromatography[16] (ECLC) still showed the presence of starting material. An additional 0.5 mL of the OsO_4 solution was added and stirring continued for 24 h when no starting material remained. Solid $NaHSO_3$ (620 mg) was added. The mixture was stirred for 10 min, and the acetone then evaporated. The residue was diluted with ethyl acetate and water, and the aqueous layer was extracted once with ethyl acetate. The combined organic extract was washed twice with brine and dried ($MgSO_4$), and the solvent was removed. After the extract was triturated and washed with ether, crystalline **12** (281 mg, 90%) was obtained, mp 136–137 °C dec. Anal. ($C_{15}H_{22}O_7$) C, H. IR: 1741 (C=O), 3436 (OH). ^{1}H NMR: The stereochemistry at C-9 is based on a NOESY cross peak between H-8a at 2.03 and H-9a′ at 3.69 ppm and conversion to **13**, below.

Epoxyartemisitene Isomers, 13 and 14. Artemisitene (200 mg, 0.71 mmol) was added to a solution of excess dimethyldioxirane in ca. 20 mL of acetone which was prepared from 50 g of Oxone according to a literature procedure.[17] After the solution was stirred overnight at room temperature, solvent was removed, and the residue was flash chromatographed on silica gel (2:1 hexane/ethyl acetate). Epoxide **13** (70 mg) eluted first, followed by **14** (80 mg). Each was recrystallized from CH_2Cl_2/ether/petroleum ether. **13**: mp 180–181 °C dec. Anal. ($C_{15}H_{20}O_6$) C, H. IR: 1747 (C=O). ^{1}H NMR: There is a NOESY cross peak between H-8a at 1.65 and H-9a′ at 2.84 ppm. **14**: mp 171–173 °C dec. Anal. ($C_{15}H_{20}O_6$) C, H. IR: 1748, 1754 (split C=O). ^{1}H NMR: There is a NOESY cross peak between H-8α at 1.79 and H-9a′ at 2.8 ppm. There is also a NOESY cross peak between 9a′ and 8β and/or 8a.

X-ray Analysis of Epoxide 14. $C_{15}H_{20}O_6$, molecular weight = 296.3, clear colorless prism (0.3 × 0.35 × 0.4), monoclinic, space group $P2_1$, a = 10.601(2) Å, b = 10.837(2) Å, c = 12.777(3) Å, β = 101.30(3)°, d_{calc} = 1.367 mg mm^{-3}, Z = 4, μ = 0.885 mm^{-1}, 2060 independent reflections were measured out to $2\theta_{max}$ = 114° with a Siemens P4 diffractometer using Cu Kα radiation (λ = 1.541 78 Å) with a graphite monchromoter in the incident beam. The data were collected at room temperature by using the ω scan technique with a variable scan rate ranging from 4°/min minimum to 30°/min maximum, depending upon the intensity of the reflection. Data were corrected for Lorentz and polarization effects. No absorption correction was applied. The structure was solved by direct methods[18] as implemented by the SHELXTL PLUS system of programs[19]. Full-matrix least-squares refinement on 378 parameters (coordinates and anisotropic thermal parameters for non-hydrogen atoms) using the 1904 reflections for which $|F_o| > 4\sigma(F_o)$. The C–H distances were fixed at 0.96 Å and placed in idealized positions. The final R factors for the configuration were R = 4.81% and R_w = 5.37%. The goodness of fit parameter was 1.05, and the final difference map was featureless.

Epoxydihydroperoxyartemisitene Isomers 15 and 16. Hydroperoxide **3** (250 mg 0.84 mmol) was added to a solution of excess dimethyldioxirane in acetone prepared from 50 g of Oxone.[17] After the solution was stirred overnight at room temperature, removal of solvent and flash chromatography (2:1 hexane/ethyl acetate) afforded 40 mg of recovered starting material, 46 mg of **15**, and 100 mg of **16**. Each epoxide was recrystallized from CH_2Cl_2/ether/petroleum ether. **15**: mp 140 °C dec. Anal. ($C_{15}H_{22}O_7$) C, H. IR: 3380, 3230 (OOH). ^{1}H NMR: stereochemistry at C-10 is based on NOESY cross peaks between the hydroperoxy hydrogen at C-10 and H-7β as well as H-12β. The stereochemistry at C-9 is based on a NOE between H-9a′ at 2.79 ppm and H-8a and between H-10 and H-9a at 3.02 ppm. **16**: mp 144–145 °C dec. Repeated combustion analysis gives a low carbon value. However, the compound analyzes for $C_{15}H_{22}O_7 \cdot 0.5\ H_2O$. IR: 3447 (OOH). ^{1}H NMR: There are NOESY cross peaks between the hydroperoxide H and 7β supporting the stereochemistry shown at position 10. There is a NOESY cross peak between H-10 at 4.96 and H-9a at 2.85 ppm.

Epoxydihydroartemisitene, 17. Alcohol **4** (100 mg, 0.35 mmol) was added to an acetone solution of excess dimethyldioxirane generated from 25 g of Oxone.[17] After the solution was stirred overnight at room temperature, solvent was removed, and the residue was flash chromatographed on silica gel (1:1 hexane/ethyl acetate) to yield 55 mg (53%) of epoxy alcohol **17**, mp 152–154 °C dec, after recrystallization from CH_2Cl_2/ether/petroleum ether. Anal. ($C_{15}H_{22}O_6$) C, H. IR: 3370 (OH).

Conversion of 15 into 13. Hydroperoxide **15** (7 mg) was added to a mixture of Ac_2O (50 μL) and pyridine (2.5 μL). After being stirred for 1 h at room temperature, the mixture was diluted with ether and water. The aqueous layer was extracted twice with ether, and the combined extract was washed with water (three times) and then brine. After drying ($MgSO_4$), solvent was removed. The residual material was identified as epoxy derivative **13** by ^{1}H NMR and by ECLC[16] retention time.

Conversion of 16 into 14. Hydroperoxide **16** (8.3 mg) was treated with Ac_2O and pyridine as above. ^{1}H NMR and ECLC identified the product as **14**.

Conversion of 16 into 17. Epoxy hydroperoxide **16** (10.4 mg, 0.033 mmol) was taken up in 0.5 mL of benzene. Triethyl phosphite (8 μL, 0.05 mmol) was added. After 7 min, the mixture was diluted with hexane. The supernatant liquid was withdrawn, and the residue was washed twice with hexane. After drying under vacuum, ^{1}H NMR of the product showed a 1:1 mixture of anomers corresponding to compound **17**, indicating that **16** and **17** have the same stereochemistry at position 9.

Conversion of 12 into 13. Diol **12** (25 mg, 0.08 mmol) was dissolved in 200 μL of pyridine. Tosyl chloride (16 mg, 0.08 mmol) was added and the mixture left at room temperature for 6 h. An additional 20 mg of TsCl was then added and the reaction mixture left in a freezer for 3 days. Several pieces of ice were added to the flask, and the product was crystallized from the aqueous mixture. The supernatant liquid was removed, and the residue was washed three times with water. After drying over P_2O_5 under vacuum, 28 mg of monotosylate was obtained. ^{1}H NMR: 7.79 d and 7.30 d (J = 8 Hz, Ar); 5.85 s (H-12); 4.50 and 4.31, AB q (J = 10.5 Hz, 9a, 9a′); 2.43 s (tosyl CH_3); 1.37 s (3-CH_3); 0.97 d (J = 5.5 Hz, 6-CH_3).

The above tosylate was dissolved in 1 mL of THF. After cooling in ice, 3 mg of imidazole was added, followed by ca. 5 mg of NaH (80% dispersion in oil). The yellow mixture was stirred at 0 °C for 30 min and then quenched with a few drops of glacial HOAc. The mixture was diluted with water and ether. The aqueous layer was extracted twice with ether, and the ether extract was then washed once with brine. After drying over $MgSO_4$ and flash chromatography on silica gel (2:1 hexane/ethyl acetate), epoxide **13** was obtained as a crystalline solid and was identified by its ECLC retention time and by ^{1}H and ^{13}C NMR.

***In Vitro* Antimalarial Studies.** The in vitro assays were conducted by using a modification of the semiautomated microdilution technique of Desjardins et al.[20] and Milhous et al.[21] as previously described.[14] Initial concentrations of test compounds were 5000 ng/mL.

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Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, and H-atom coordinates for compound **14** and a table of ^{1}H-NMR data including coupling constants for compounds **1**, **2**, and **5**–**17** (8 pages). Ordering information is given on any current masthead page. Tables of atomic coordinates

and bond lengths and angles have been deposited with the Crystallographic Data Centre, Cambridge University Chemical Laboratory, Cambridge CB2 1EW, U.K.

References

(1) Looareesuwan, S.; Viravan, C.; Vanijanouta, S.; Wilairatana, P.; Suntharasamai, P.; Charoenlarp, P.; Arnold, K.; Kyle, D.; Canfield, C.; Webster, K. Randomized trial of artesunate and mefloquine alone and in sequence for acute uncomplicated falciparum malaria. *Lancet* **1992**, *339*, 821–824.

(2) Reports from the XIII International Congress for Tropical Medicine and Malaria, Jomtien, Pattaya, Thailand, 29 Nov–4 Dec 1992: Hien, T. T.; Arnold, K. Abstract no. MoS8-1; Li, G.-Q. no. MoS8-2; Win, K. no. MoS8-3; Bunnag, D.; Karbwang, J.; Chittamas, S.; Harinasuta, T. no. MoS8-5; Fu, Y.-J.; Jia, J. no. MoP11-3; Cai, X.; Tang, X. no. MoP11-4; Wang, H.; Shi, W.; Feng, T.; Le, J.; Zhang, S.; Li, P.; Li, G.; Guo, X. no. MoP11-7; Wang, W.-L.; Ahang, J. C.; Fu, Y. X.; Deng, X. L.; Fu, L. C.; Guo, X. B. no. MoP11-6; Vinh, H.; Arnold, K.; Cuong, B. M.; Phu, N. H.; Chau, T. T. H.; Hoa, N. T. M.; Chuong, L. V.; Mai, N. T. H.; Vinh, N. N.; Trang, T. T. M. no. MoP11-10.

(3) Zaman, S. S.; Sharma, R. P. Some Aspects of the Chemistry and Biological Activity of Artemisinin and Related Antimalarials. *Heterocycles* **1991**, *32*, 1593–1637.

(4) Butler, A. R.; Wu, Y.-L. Artemisinin (Qinghaosu): A New Type of Antimalarial Drug. *Chem. Soc. Rev.* **1992**, 85–90.

(5) Acton, N.; Klayman, D. L. Artemisitene, a New Sesquiterpene Lactone Endoperoxide from *Artemisia annua*. *Planta Medica* **1985**, 441–442.

(6) El-Feraly, F. S.; Ayalp, A.; Al-Yahya, M. A.; McPhail, D. R.; McPhail, A. T. Conversion of Artemisinin to Artemisitene. *J. Nat. Prod.* **1990**, *53*, 66–71.

(7) El-Feraly, F. S.; Ayalp, A.; Al-Yahya, M. A.; McPhail, D. R.; McPhail, A. T. Decomposition of Dihydroartemisitene on Silica Gel. *J. Nat. Prod.* **1990**, *53*, 920–925.

(8) Blasko, G.; Cordell, G. A. Definitive ^{1}H- and ^{13}C-NMR Assignments of Artemisinin (Qinghaosu). *J. Nat. Prod.* **1988**, *51*, 1273–1276.

(9) The numbering system used for artemisinin (Registry no. 63968-64-9) is that preferred by Chemical Abstracts. However, in Figure 1, the oxygen atoms are numbered consecutively from 1 to 6 for clarity.

(10) Lin, A. J.; Li, L.-Q.; Klayman, D. L.; George, C. F.; Flippen-Anderson, J. L. Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives. 3. Aromatic Amine Derivatives. *J. Med. Chem.* **1990**, *33*, 2610.

(11) Acton, N.; Klayman, D. Conversion of Artemisinin (Qinghaosu) to *Iso*-Artemisitene and to 9-*Epi*-Artemisinin. *Planta Medica* **1987**, 266–268.

(12) Avery, M. S.; Jennings-White, C.; Chong, W. K. M. Simplified Analogues of the Antimalarial Artemisinin: Synthesis of 6,9-Desmethylartemisinin. *J. Org. Chem.* **1989**, *54*, 1792–1795.

(13) Lin, A. J.; Li, L.-Q.; Milhous, W. K.; Klayman, D. L. Antimalarial Activity of Dihydroartemisinin Derivatives. 4. Stereoselectivity of 9-Hydroxy Esters. *Med. Chem. Res.* **1991**, *1*, 20–23.

(14) Lin, A. J.; Li, L.-Q.; Andersen, S. L.; Klayman, D. L. Antimalarial activity of New Dihydroartemisinin Derivatives. 5. Sugar Analogues. *J. Med. Chem.* **1992**, *35*, 1639–1642.

(15) Vennerstrom, J. L.; Eaton, J. W. Oxidants, Oxidant Drugs, and Malaria. *J. Med. Chem.* **1988**, *31*, 1269–1277.

(16) Acton, N.; Klayman, D. L.; Rollman, I. J. Reductive Electrochemical HPLC Assay for Artemisinin (Qinghaosu). *Planta Medica* **1985**, *445*–446.

(17) Adam, W.; Chan, Y.-Y.; Cremer, D.; Gauss, J.; Scheutzow, D.; Schindler, M. Spectral and Chemical Properties of Dimethyldioxirane as Determined by Experiment and ab Initio Calculations. *J. Org. Chem.* **1987**, *52*, 2800–2803.

(18) Karle, J.; Karle, I. L. The Symbolic Addition Procedure for Phase Determination for Centrosymmetric and Noncentrosymmetric Crystals. *Acta Crystallogr.* **1966**, *21*, 849–859.

(19) Sheldrick, G. M. Crystallographic Algorithms for Mini and Maxi Computers. In *Crystallographic Computing*; Sheldrick, G. M., Krüger, C., Goddard, R., Eds.; Oxford University Press: Oxford, 1985; Vol. 3, pp 175–179.

(20) Desjardins, R. E.; Canfield, C. J.; Haynes, D. E.; Chulay, J. D. Quantitative Assessment of Antimalarial Activity in vitro by a Semiautomated Microdilution Technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.

(21) Milhous, W. K.; Weatherley, N. F.; Bowdre, J. H.; Desjardins, R. E. *In Vitro* Activities of and Mechanism of Resistance to Antifol Antimalarial Drugs. *Antimicrob. Agents Chemother.* **1985**, *27*, 525–530.